510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
K033727

B. Purpose for Submission:
Labeling changes, Indications for Use modified

C. Analyte:
Parathyroid hormone,

D. Type of Test:
Quantitative

E. Applicant:
Nichols Institute Diagnostics

F. Proprietary and Established Names:
Nichols Advantage® Bio-Intact PTH (1-84) Immunoassay

G. Regulatory Information:

1. Regulation section:
   862.1545, Parathyroid hormone test system
2. Classification:
   Class II
3. Product Code:
   CEW
4. Panel:
   75

H. Intended Use:

1. Intended use(s):
   The Nichols Advantage® Bio-Intact PTH (1-84) assay is intended for use with the Nichols Advantage® Specialty System to measure the levels of parathyroid hormone in serum, EDTA plasma and heparinized plasma. Measurements of parathyroid hormone levels are used in the differential diagnosis of hypercalcemia (abnormally high levels of calcium in the blood) and hypocalcemia (abnormally low levels of calcium in the blood) resulting from disorders of calcium metabolism. Measurements of parathyroid hormone levels with Bio-Intact PTH (1-84) are also used as an aid in monitoring therapeutic intervention of secondary hyperparathyroidism that frequently occurs in chronic kidney disease. Assay results should be used in

conjunction with other clinical data to assist the clinician in making individual patient management decisions.

2. Indication(s) for use:
   See Intended Use above
3. Special condition for use statement(s):

4. Special instrument Requirements:
   Nichols Advantage® Specialty System

I. Device Description:

One (1) cartridge contains the following reagents sufficient for 100 tests:

1. Bio-Intact PTH (1-84) Magnetic Particles
   One vial (2.8 mL) containing streptavidin-coated magnetic particles in PBS buffer and ≤0.05% sodium azide and ProClin® 300 as preservatives.
2. Acridinium Ester-labeled Antibody Solution
   One vial (3.0 mL) containing acridinium ester-labeled anti-Bio-Intact PTH antibody, BSA and ProClin-300® as preservative in a PBS buffer.
3. Biotinylated Antibody Solution
   One vial (7.9 mL) containing biotinylated anti-Bio-Intact PTH antibody, BSA, other proteins, and ≤0.095% sodium azide as preservative in a PBS buffer.
4. Diluent
   One vial (8.0 mL) containing buffered protein solution and ≤0.095% sodium azide as preservative.
5. One lot specific Bio-Intact PTH (1-84) Master Curve Bar Code Card

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Nichols Advantage® Bio-Intact PTH (1-84) Immunoassay
2. Predicate K number(s):
   K013992
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Test principle	Immunochemiluminometric sandwich assay	Same
Tracer	Acridium ester-labeled goat polyclonal anti-intact PTH antibody	Same
Separation system	Streptavidin-coated magnetic particles	Same
Differences		
Item	**Device**	**Predicate**

Indications for Use	The Nichols Advantage® Bio-Intact PTH (1-84) assay is intended for use with the Nichols Advantage® Specialty System to measure the levels of parathyroid hormone in serum, EDTA plasma and heparinized plasma. Measurements of parathyroid hormone levels are used in the differential diagnosis of hypercalcemia (abnormally high levels of calcium in the blood) and hypocalcemia (abnormally low levels of calcium in the blood) resulting from disorders of calcium metabolism. Measurements of parathyroid hormone levels with Bio-Intact PTH (1-84) are also used as an aid in monitoring therapeutic intervention of secondary hyperparathyroidism that frequently occurs in chronic kidney disease. Assay results should be used in conjunction with other clinical data to assist the clinician in making individual patient management decisions.	The Nichols Advantage® Bio-Intact PTH (1-84) assay is intended for use with the Nichols Advantage® Specialty System to measure the levels of parathyroid hormone in serum and EDTA plasma. Measurements of parathyroid hormone levels are used in the differential diagnosis of hypercalcemia (abnormally high levels of calcium in the blood) and hypocalcemia (abnormally low levels of calcium in the blood) resulting from disorders of calcium metabolism. Assay results should be used in conjunction with other clinical data to assist the clinician in making individual patient management decisions.
Sample types	Serum, EDTA plasma, heparin plasma	Serum, EDTA plasma
Sample collection and storage instructions	EDTA and heparinized plasma may be stored refrigerated (2-8 ºC) up to 48 hours. EDTA whole blood may be stored refrigerated (2-8 ºC) up to 48 hours before centrifugation. Serum should be frozen within 2	Centrifuge samples and freeze serum and plasma immediately. EDTA and heparin plasma should be assayed within two hours once placed on the system.

	hours. EDTA plasma and heparin plasma may be exposed to room temperature up to 6 hours during testing.	

K. Standard/Guidance Document Referenced (if applicable):
NCCLS EP5-A

L. Test Principle:
The Bio-Intact PTH (1-84) assay is a two-site immunochemiluminometric assay. Two goat polyclonal antibodies are used. One antibody is biotin labeled and used as capture and the second antibody is labeled with acridinium ester and used for detection. The sample is added to a cuvette followed by addition of the two anti-PTH antibodies and the streptavidin-coated magnetic particles. The reaction mixture is allowed to incubate for 30 minutes at 37 ºC. Because of the high affinity interaction between biotin labeled antibody and streptavidin, the sandwich complex is captured onto the streptavidin magnetic particles. The captured complex bound to the magnetic particles is then washed by the system to remove unbound components and acridinium-ester labeled antibody. The cuvette wells containing the washed magnetic particles are transported into the system luminometer, which automatically injects Trigger 1 and Trigger 2, initiating the chemiluminescence reaction. The light is quantitated by the luminometer and expressed as RLU. The amount of bound-labeled antibody is directly proportional to the concentration of intact PTH in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:
The within-run and total imprecision performance for the Bio-Intact PTH (1-84) assay was estimated using the NCCLS EP5-A method (Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline). The data represent two runs per day over 20 days with each serum pool run in duplicate. The study was performed on a single Nichols Advantage system.

Control Pool	Mean (pg/mL)	Within-Run		Total Imprecision	
		SD	%CV	SD	%CV
A	5.5	0.3	5.5	0.6	10.9
B	10.3	0.4	3.9	0.9	8.7
C	24.8	0.7	2.8	1.5	6.0
D	276	6	2.2	14	5.1

b. Linearity/assay reportable range:
The highest reportable value without dilution is the value of the highest point on the Master Curve (1800 pg/mL). Samples reading above the Master Curve should be diluted and repeated, or reported as greater than the highest value on the Master Curve. Samples with

varying concentrations of Bio-Intact PTH (1-84) were either manually diluted with Sample Diluent before placing onto the system, or diluted on-board the system. The results demonstrate linearity across the range of the assay.

c. Traceability (controls, calibrators, or method):
The PTH standards are prepared analytically on a mass basis from purified synthetic intact PTH.

d. Detection limit:
Based upon an inter-assay precision that approximates 20% CV, the Limit of Quantitation (LOQ, functional sensitivity) is estimated to be less than 4.0 pg/mL. Values below 4.0 pg/mL should be reported as "less than 4.0 pg/mL" (<4.0 pg/mL).

e. Analytical specificity:
The Bio-Intact PTH (1-84) Assay was tested for crossreactivity for the 7-84 PTH fragment up to a level of 3000 pg/mL. No detectable crossreactivity was observed. PTH fragments 39-68, 53-84, 44-68, 39-84 minimally interfered. Bilirubin up to 20 mg/dL, triglycerides up to 3000 mg/dL, hemoglobin up to 200 mg/dL and protein up to 6000 mg/dL do not interfere in the assay defined as recovery within ± 10% of control value. Hemolyzed samples greater than 200 mg/dL may give over recovery. Such samples should be avoided. Grossly lipemic, hemolyzed, icteric and samples containing high levels of total protein can affect immunological reactions and should not be run.

f. Assay cut-off:
NA

2. Comparison studies:

a. Method comparison with predicate device:
The Bio-Intact PTH (1-84) Assay (Y) was compared to the Nichols Advantage Intact PTH Chemiluminescence assay (X). Three hundred five (305) serum samples were assayed by both methods. The range of values observed with the Intact PTH was 5.0 to 1387 pg/mL; with the Bio-Intact PTH (1-84) Assay the range was 3.0 to 746 pg/mL. Passing Bablok regression analysis of these data yielded an equation of $Y = 0.66X – 0.6$ (95% confidence intervals of the slope and intercept were 0.64 to 0.68 and –1.1 to –0.2 respectively). Least Squares Linear Regression analysis of these data yielded an equation of $Y = 0.60X + 4.2$ (95% confidence intervals of the slope and intercept were 0.58 to 0.62, and + 1.9 to + 6.5 respectively). Pearson's correlation coefficient (r) of the paired data was 0.97 (95% confidence interval is 0.96 to 0.98). The Bio-Intact PTH (1-84) Assay (Y) was also compared to the Nichols Advantage Intact PTH Chemiluminescence assay (X) with split samples from patients on dialysis and renal care. A linear regression analysis of results from 3199 dialysis samples gave an equation of $Y=0.52 + 1.2$ (95% confidence interval of the slope and intercept were 0.515 to 0.523

and –0.620 to 3.119 respectively) with an r of 0.97 (95% confidence interval is 0.975 to 0.978).

b. Matrix comparison:

To study whether different specimen types can be used to measure Bio-Intact PTH, blood was drawn from n=27 adult volunteers. The following Becton Dickinson brand Vacutainer tubes were used: Red Top silicone coated interior, plain no additives; SST gel separator with clot activator – silicone coated interior; EDTA with 15% K3-EDTA – silicone coated interior; EDTA with 15% K3-EDTA no interior coating and Heparin containing 143 USP units of sodium heparin no interior coating. After blood was drawn, blood was processed using a refrigerated centrifuge, placed on ice with serum and plasma rapidly aliquoted and frozen at –70 ºC. In all cases serum or plasma was frozen within 2 hours of venipuncture. On the day of the assay, an aliquot of sera and plasma from each specimen tube was thawed at the same time and assayed within the same run. The chart describes the mean and standard deviation of the observed results. At the 95% confidence level, there was no significant difference in Bio-Intact PTH results between specimen tube types.

Tube Type	Red Top (plain)	SST gel separator	EDTA silicone coated	EDTA (Plain)	Heparin
Mean ± sd (pg/mL)	23.1 ± 10.5	24.1 ± 10.8	22.1 ± 10.3	22.0 ± 9.7	22.6 ± 10.2

3. Clinical studies:

a. Clinical sensitivity:

NA

b. Clinical specificity:

NA

c. Other clinical supportive data (when a and b are not applicable):

- Stability studies were performed on different tubes under different conditions, using the Nichols Advantage Bio-Intact PTH (1-84) Assay.

Table 1: Summary of results of stability studies with whole blood performed at four sites. Samples after draw were kept at refrigerated conditions without centrifugation. Aliquots were processed for plasma at periodic intervals, and tested.

Site	Sample source	Whole blood tubes	n	**Mean recovery**				
				0 hr	8 hrs	24 hrs	36 hrs	48 hrs
1	Dialysis Lab	EDTA	41	100%	99%	99%	99%	99%
2	Dialysis Lab	EDTA PPT	72	100%				99%
3	Dialysis Lab	EDTA	40	100%	99%	102%		99%
4	Volunteer donors	EDTA	30	100%			105%	102%

Table 2: Stability studies at refrigerated conditions with EDTA and heparin plasma.

Sample Type	Mean recovery			
	0 hr	24 hrs	36 hrs	48 hrs
EDTA plasma	100%	97%	99%	99%
Heparin plasma	100%	98%	101%	98%

Table 3: Stability studies at room temperature with EDTA and heparin plasma.

Sample Type	Mean recovery	
	0 hr	8 hrs
EDTA plasma	100%	101%
Heparin plasma	100%	94%

The following specimen collection, preparation and storage instructions were established based on the results of the studies and are included in the labeling:

EDTA whole blood (with or without gel separator) can be handled and stored at refrigerated conditions (2-8 °C) before centrifugation to separate cells for plasma. Alternately, EDTA whole blood may be centrifuged promptly after collection and the separated plasma can be stored at refrigerated conditions. Heparinized blood should be centrifuged promptly after collection and the separated plasma can be stored at refrigerated conditions. In all these instances, testing should be completed in less than 48 hours after collection. Therefore EDTA whole blood stored refrigerated can be centrifuged immediately before testing or any time within 48 hours of draw, with the plasma assayed immediately. Heparinized gel separation tubes have not been tested and are not recommended. Sera should be rapidly processed and frozen within 2 hours of collection. Frozen sera should be thawed rapidly and tested promptly.

Handle and process blood preferably under refrigerated conditions. Centrifuge the specimen, preferably in a refrigerated centrifuge. Separate plasma from the cells avoiding hemolyzed and lipemic samples. Hemolysis may indicate the specimen may have been mistreated during handling or during the collection process.

Plasma can be frozen (-20 °C or colder in a non-defrosting freezer) for longer-term storage. Samples should not be subjected to multiple freeze and thaw cycles, preferably not more than one such cycle. If a sample needs to be tested at multiple times, multiple frozen aliquots of specimens are preferred. Occasionally samples with unusual constitution, specific for the sample, may exhibit atypical stability characteristics.

On the day the assay is to be performed, frozen specimens should be rapidly thawed at room temperature, thoroughly mixed and centrifuged

prior to placement onto the instrument. Centrifugation is recommended because plasma will shed fibrin after freezing and thawing. EDTA plasma and heparin plasma may be exposed to room temperature up to 6 hours during the course of normal laboratory operations for testing. It is recommended that once specimens are loaded on the system, they be assayed immediately.

- The sponsor also provides 23 references to support the claim which has been added to the Indications for Use that "Measurements of parathyroid hormone levels with Bio-Intact PTH (1-84) are also used as an aid in monitoring therapeutic intervention of secondary hyperparathyroidism that frequently occurs in chronic kidney disease."

4. Clinical cut-off:
   NA
5. Expected values/Reference range:
   To establish an expected reference range, sera from n=336 healthy adult blood bank volunteers (18-66 years) was obtained in Belgium. Per Belgium's regulatory requirements, individuals donating blood undergo physical examination and have a health history taken. At the time of blood donation, an extra tube of blood was obtained for PTH testing. Additional biochemical testing was performed to exclude individuals with an abnormal hemoglobin level, or abnormal serum calcium, creatinine, or 25-hydroxyvitamin D level. Because of seasonal variation of 25-hydroxyvitamin D, blood was obtained during two time periods: the end of winter (March 2003) and the end of summer (September 2002). The winter cohort totaled n=180 adults, and consisted of 85 females, and 95 males. The summer cohort totaled n=156 adults, and consisted of 87 females, and 69 males. No age related influence on levels of Bio-Intact PTH, either by gender or by season was observed. In this study, seasonal variation of Bio-Intact PTH was observed. Furthermore, male levels were slightly higher than female levels. This study demonstrates that normative Bio-Intact PTH levels were associated with gender, seasonal variation of 25-hydroxyvitamin D levels, and the geographic location of volunteers. The higher PTH levels observed in winter were associated with lower serum 25-hydroxyvitamin D levels in the healthy cohort. For these reasons, interpretation of normative PTH levels should take these factors into account. The data were normally distributed after log transformation. The geometric mean and 95% confidence intervals obtained in this study are as follows.

Season (n)	Geometric Mean (pg/mL)	95% CI (pg/mL)
Winter + Summer (n=336)	19	8 - 50
Winter (n=180)	25	12 - 52
Summer (n=156)	15	7 - 32

N. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.